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TITLE: Cadherin-11 Regulation of Fibrosis through Modulation of Epithelial-to-Mesenchymal Transition: Implications for Pulmonary Fibrosis in Scleroderma

PRINCIPAL INVESTIGATOR: Sandeep K. Agarwal, M.D., Ph.D.

CONTRACTING ORGANIZATION: Baylor College of Medicine  
Houston, TX 77030-3411

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14. ABSTRACT Lung fibrosis is the leading cause of death in scleroderma. Treatment options are limited, stressing the unmet need to advance of understanding of the. We have demonstrated that cadherin-11 (Cad11) is increased in fibrotic skin and lung tissues and that Cad11 is a mediator of fibrosis in mouse models. Mechanistically how this occurs is not known, but our preliminary data point to a role for Cad11 in the regulation of epithelial to mesenchymal transition. In year 1 of the grant, we have performed experiments in the intraperitoneal model of pulmonary fibrosis in wild type and Cad11 deficient mice that show that Cad11 deficient mice have less lung fibrosis. Initial studies studies also suggest that antiCad11 antibodies are effective in treating lung fibrosis in this model. In vitro studies have demonstrated that Cad11 regulates epithelial-to-mesenchymal-transition (EMT) in MLE-12 cells, a mouse alveolar epithelial cell line. These data are confirming our original hypothesis that Cad11 regulates lung fibrosis through modulation of EMT. Finally, in year 1, we identified the patient sera that will be used in year 2 to determine soluble circulating levels of Cad11.					
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## **Progress Report Summary (October 2014, Year 2).**

Grant DoD Award W81XWH-12-1-0516, Cadherin-11 Regulation of Fibrosis through Modulation of Epithelial-to-Mesenchymal Transition: Implications for Pulmonary Fibrosis in Scleroderma

### **INTRODUCTION.**

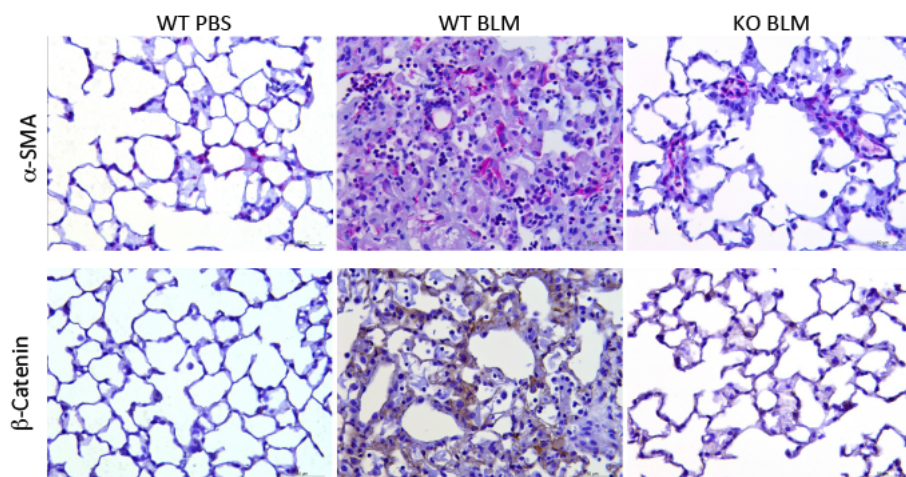
My laboratory focuses on the potential role of cadherin-11 (Cad11) in fibrosis. We have previously reported that Cad11 expression is increased in fibrotic tissues from lungs of patients with idiopathic pulmonary fibrosis and skin of patients with systemic sclerosis. Subsequent studies have demonstrated that Cad11 is a critical mediator of lung and skin fibrosis using the intratracheal (IT) and subcutaneous bleomycin models. Preliminary studies suggest that Cad11 may regulate type 2 alveolar epithelial cell epithelial-to-mesenchymal transition (EMT), a process that contributes to the development of lung fibrosis. As opposed to the IT bleomycin lung fibrosis model, repeated administration of bleomycin via the intraperitoneal (IP) route is considered to better mimic human lung fibrosis and the process of EMT. This proposal builds on these recent observations and utilizes the IP bleomycin pulmonary fibrosis model. We hypothesize that Cad11 regulates the EMT in AEC during the development of pulmonary fibrosis and that cadherin-11 is therapeutic target in the intraperitoneal bleomycin model of pulmonary fibrosis. This proposal will be the first identify novel mechanisms by which Cad11 regulates EMT and build the foundation for additional translational studies seeking to develop Cad11 as a therapeutic target for SSc-ILD.

### **BODY**

#### **RESEARCH RESULTS**

**Specific Aim 1.** Determine the role of cadherin-11 in the intraperitoneal bleomycin model of pulmonary fibrosis and the extent to which cadherin-11 modulates epithelial-to-mesenchymal transition in vivo.

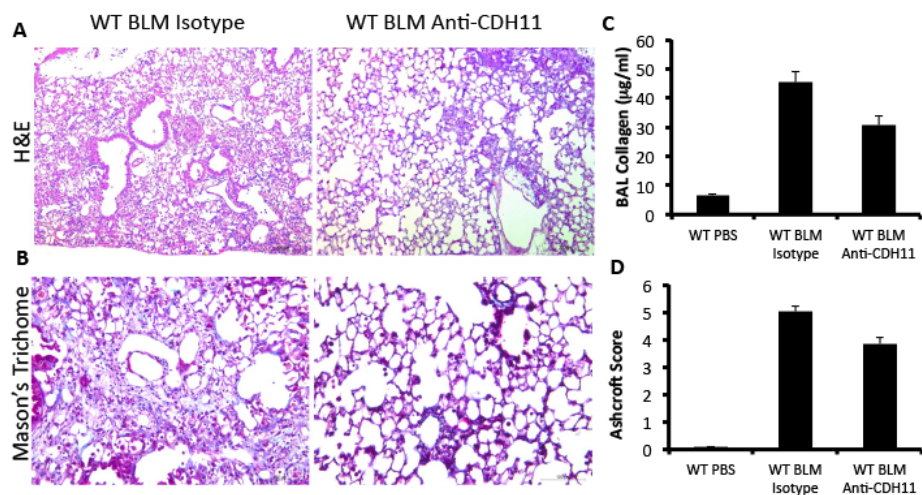
In the first year of the proposal, we have performed the intraperitoneal pulmonary fibrosis model in wild type and cadherin-11 deficient mice. These data demonstrated that cadherin-11 deficient mice had decrease subpleural fibrosis when administered bleomycin (BLM) compared to wild type mice. The data presented in year 1 include histology, oxygen saturation levels in the blood and BAL cell counts. In year 2, we have extended these data to show that the levels of alpha smooth muscle actin was also decreased. Alpha smooth muscle actin is a marker of the myofibroblast, the major producer of extracellular matrix in the development of pulmonary fibrosis. These data indicate that in the absence of cadherin-11 the number of myofibroblasts that



**Figure 1. Pulmonary phenotype following intraperitoneal bleomycin exposure in Cad11 deficient mice.** This figure displays representative immunohistochemistry of lungs from wild type (WT) mice treated with PBS (left) and bleomycin (BLM, middle). Cad11 deficient mice (KO) exposed to BLM are shown in the right panels. Examination of lung histology through alpha smooth muscle actin IHC (top) and beta-catenin IHC (bottom) revealed that KO mice exposed to bleomycin displayed a reduction in myofibroblasts and beta catenin expression. n= 8 mice per group

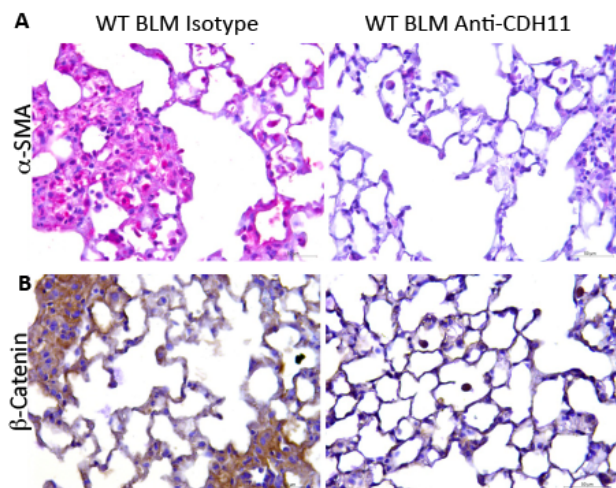
are present in the lung is decreased. In addition previous data have supported an important role for beta catenin in the development of pulmonary fibrosis and skin fibrosis. Beta catenin IHC was performed and demonstrated that beta-catenin expression was lower in cadherin-11 deficient mice.

In year 2, we extended the observations in cadherin-11 deficient mice by performing experiments using the antiCad11 monoclonal antibodies as a therapeutic agent in wild type mice given IP BLM. In these experiments, treatment with isotype control or antiCad11 antibodies were started in the 3<sup>rd</sup> week of the 4 week model. As seen in figure 2, antiCad11 antibody treatment reduced lung fibrosis as assessed using histology, Ashcroft scores and BAL collagen levels.



**Fig 2. Treatment of pulmonary fibrosis with antiCad11 monoclonal antibodies.** (A) H&E and (B) Masson's trichrome staining of lung sections demonstrates less fibrosis in mice treated with antiCDH11 antibody, which was quantified in (D) using Ashcroft scoring. (C) Soluble collagen protein levels were measured using Sircol Assay. Data presented as mean mg collagen/ml BAL fluid. (D) Ashcroft scores were used to determine the degree of fibrosis. Data are presented as mean  $\pm$  SEM. (\*,  $p \leq 0.05$  WT PBS vs. WT BLM and #,  $p \leq 0.05$  WT BLM vs. KO BLM; n=8 mice per group).

Additional analyses of anti-cadherin-11 antibody treated wild type mice in the IP BLM model also demonstrate a decrease in myofibroblasts (alpha smooth muscle actin IHC) and beta catenin levels (figure 3). These data indicate that anti-cadherin-11 antibodies are effective in treating pulmonary fibrosis.



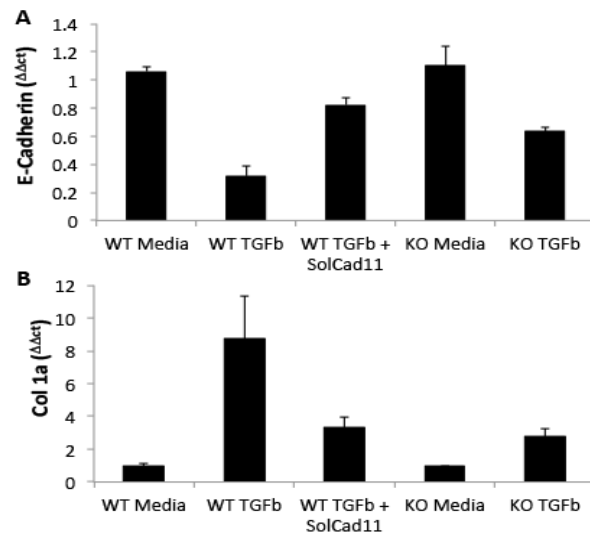
**Fig 3. Treatment of pulmonary fibrosis with antiCad11 monoclonal antibodies.** This figure displays representative immunohistochemistry of lungs from wild type (WT) mice exposed to IP BLM and treated with antiCDH11 antibodies. AntiCDH11 antibodies decreased alpha smooth muscle actin expression and beta-catenin expression as determined by IHC.

Finally, in year 3, we have acquired the SP-C-Cre, Rosa26 lacZ reporter mice and the breeding colony has been started for experiments in years 3. In year 3, we will determine the expression of EMT markers in these tissues by qRTPCR and IHC.

**Specific Aim 2.** Determine the contribution of cadherin-11 to process of epithelial-to-mesenchymal transition (EMT) and modulation of Rho-GTPases in airway epithelial cells (AECs) in vitro.

In year 1, we demonstrated that cadherin-11 regulated EMT in immortalized alveolar epithelial cell (AEC) lines. In year 2, we extended these observations utilizing primary AECs isolated from wild type and cadherin-

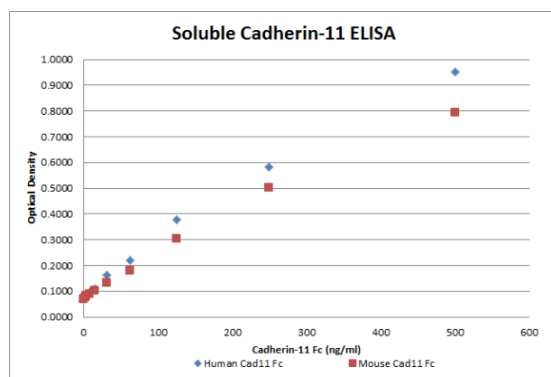
11 deficient mice. Primary AECs from wild type and cadherin-11 deficient mice were isolated and cultured with TGF-beta to drive the process of EMT. During EMT, E-cadherin decreases and collagen increases, therefore these mRNA transcripts were used for quantifying EMT. The results of these experiments are provided in figure 4. TGF-beta decreased E-cadherin expression and increased collagen expression in wild type AECs, which was attenuated by soluble cadherin-11 fusion protein (which inhibits cadherin-11 function). In addition, in comparison to wild type AECs, primary AECs isolated from cadherin-11 deficient mice had a reduction in the changes in E-cadherin and collagen expression induced by TGF-beta. Together with the data from year 1 in MLE-12 cells, these new data indicate that cadherin-11 is a regulator of epithelial-mesenchymal transformation in alveolar epithelial cells. In years 3, we will perform additional analyses of EMT in these cells.



**Figure 4. Cadherin-11 regulates EMT in vitro in primary alveolar epithelial cells.**

**Specific Aim 3.** Determine the circulating levels of cadherin-11 in scleroderma patients with interstitial lung disease.

We have been working with our collaborators at UTHSC to identify and sera that will be used for our soluble Cad-11 ELISA. The SSc samples have been identified and aliquotted for our ELISA detection. These include 299 patients (83% female, avg age 49, avg disease duration 2.5 years, 59% with diffuse SSc, 28% with ILD and avg skin score of 16 at enrollment). Since there is not a commercial Cad-11 ELISA, we have optimized our conditions using 2 anti-Cad-11 antibodies (clones 3H10 and 23C6). As seen in figure 5, our ELISA can detect both human and mouse soluble Cad-11. In year 3, we will determine the circulating levels of Cad-11 in scleroderma patients and controls as proposed in the original Aim 3.



**Figure 5. Soluble Cad-11 ELISA detects human (blue) and mouse (red) Cad-11**

## **KEY RESEARCH ACCOMPLISHMENTS**

1. Cadherin-11 deficient mice have decrease pulmonary fibrosis and decrease numbers of myofibroblasts in the intraperitoneal model of pulmonary fibrosis
2. AntiCad11 antibodies are effective in treating lung fibrosis and reducing the number of myofibroblasts in the intraperitoneal model of pulmonary fibrosis this model
3. Cad11 regulates the in vitro TGF-beta induced epithelial-to-mesenchymal-transition (EMT) in MLE-12 cells, a mouse alveolar epithelial cell line and primary alveolar epithelial cells.
4. Systemic sclerosis patient sera have been identified and aliquotted to be used in year 3 to determine soluble circulating levels of Cad11.

## **REPORTABLE OUTCOMES.**

1. Abstract and Poster Presentation at the 2013 American College of Rheumatology Annual Meeting in San Diego, CA.  
Ref : “Pedroza M, George AT, Agarwal SK. Cadherin-11 regulates pulmonary fibrosis in bleomycin lung injury. Arthritis and Rheumatism. 65(S10):S1095, 2013.”

## **CONCLUSIONS**

Cadherin-11 is a mediator of lung fibrosis and can regulate epithelial-to-mesenchymal-transition (EMT) in MLE-12 cells, a mouse alveolar epithelial cell line.

## **REFERENCES**

None for current report

## **APPENDICES**

None for current report

## **SUPPORTING DATA**

No additional data for current report, see “BODY” section above for data.